Supplementary material for the manuscript:

Mammalian MT1 and MT2 metallothioneins differ in their metal binding abilities

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Material and Methods. Detailed information of the experimental methodology indicated in the main text.

Figure S1. Deconvoluted ESI-MS spectra corresponding to the titration of a 10 µM solution of the Zn-mMT2 preparation with Cu(I) carried out at neutral pH and analyzed at both, neutral and acidic pH.

Figure S2. Deconvoluted ESI-MS spectra corresponding to the titration with Cu(I) of (A) a 20 µM solution of Zn_4-αmMT2 and (B) a 20 µM solution of Zn_3-βmMT2, both carried out at neutral pH and analyzed at neutral and acidic pH.

Material and Methods

Synthesis and purification of the recombinant metal-MT complexes. To overexpress MT, the recombinant constructs were introduced into the protease deficient strain *E.coli* BL21. Overnight cultures of the transformed host strains in LB supplemented with 100 mg l^{-1} ampicillin at 37 °C were inoculated to 1.5 L of fresh LB medium. After growth at 37 °C to an A600 value of 1.0, protein synthesis was induced with 100 mM IPTG (isopropyl-β-D-thiogalactopyranoside) final concentration, 30 min before the addition of the desired metal salt solution (300 μM $ZnCl_2$, 300 μM $CdCl_2$, or 500 μM $CuSO_4$, final concentration). Copper cultures were performed under two aeration conditions: normal or regular aeration (1-L culture in 2.5-L Erlenmeyer flasks at 220 rpm agitation), and low aeration (1.5-L culture in 2.5-L Erlenmeyer flasks at 150 rpm agitation). Bacterial growth was allowed for an additional 2.5 h and then the cell biomass was harvested and washed in PBS (phosphate-buffered saline, 1.4 M NaCl, 27 mM KCl, 101 mM Na_2HPO_4, 18 mM KH_2PO_4) and pelleted by centrifugation at 7700 x *g* for 10 min. For protein purification, cells were resuspended in 5% of the original volume of ice-cold PBS-0.5% v:v β-mercaptoethanol. To prevent oxidation, pure grade 5.6 argon was bubbled through all the steps of the purification procedure. Suspended cells were sonicated at 4 °C with 20 s pulses for 5 min, and centrifuged at 12000 x *g* for 30 min. The supernatant was recovered and used to purify the GST-MT and fusion protein by batch affinity chromatography with Gluthatione-Sepharose-4B at a volume ratio 1:10 matrix:sample. The mixture was incubated with gentle agitation for 60 min at room temperature. After three washes in PBS, a thrombin-PBS solution (10 units of thrombin/mg of fusion protein) was poured into the matrix bed, and digestion was carried out overnight at 23–25 °C. The GST portion of the expressed fusion protein remained bound to the gel matrix, whereas the MT portion remained in solution together with thrombin. The supernatant was then five-fold concentrated using Centriprep Concentrators (Amicon) with a cutoff of 3 kD, and subsequently fractionated using FPLC. A Superdex-75 exclusion column was equilibrated with 50 mM Tris-HCl, pH 7.0 and run at 1 ml min^{-1}. 1 ml fractions were collected and analysed for protein content by their absorbance at 254 and 280 nm. Aliquots of the protein-containing Superdex-75 fractions were analysed by SDS-PAGE on 15% gels and stained with Coomassie Blue. Positive samples were pooled, and aliquots were stored at -70 °C for further use.

***In vitro* prepared metal-MT complexes:**

Metal replacement titrations (Zn/Cd and Zn/Cu) were performed by adding the correspondent metal ions at equivalent molar ratios to the recombinant Zn-MT complexes at pH 7.0 under strict anaerobic conditions. The following solutions were used: $CdCl_2$ in water, MERCK AAS Cd^{2+} standard of 1000 ppm, or Cu^+ ($[Cu(CH_3CN)](ClO_4)$ in 30% v/v CH_3CN/H_2O).

Figure S1. Deconvoluted ESI-MS spectra corresponding to the titration of a 10 µM solution of the Zn-mMT2 preparation with Cu(I) carried out at neutral pH and analyzed at both, neutral and acidic pH.

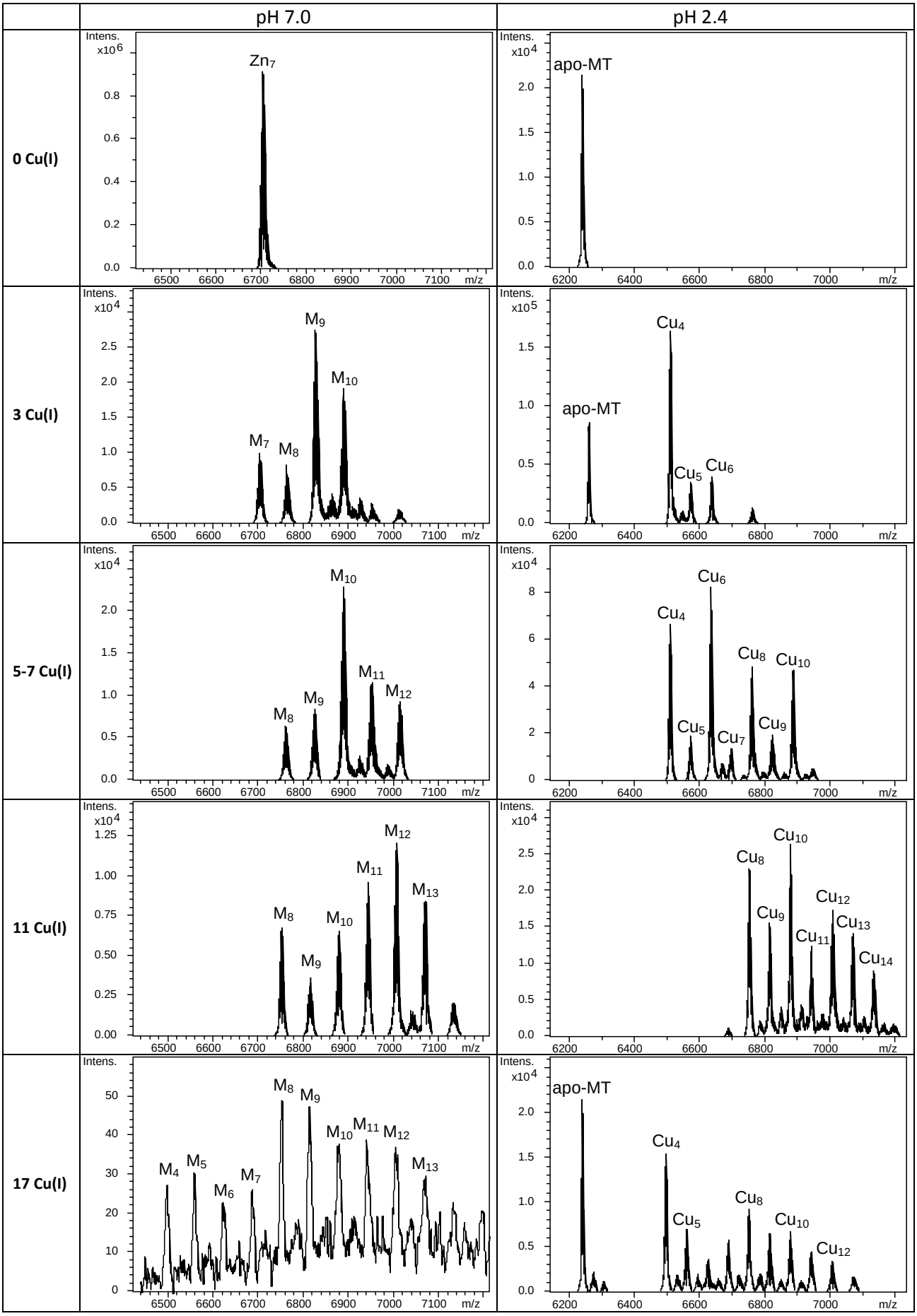

Figure S2. Deconvoluted ESI-MS spectra corresponding to the titration with Cu(I) of (A) a 20 µM solution of Zn_4-αmMT2 and (B) a 20 µM solution of Zn_3-βmMT2, both carried out at neutral pH and analyzed at neutral and acidic pH.